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Supporting Information

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A Panel of Cytochrome P450 BM3 Variants To Produce Drug Metabolites and Diversify Lead Compounds

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Supplementary Table 1. Identity of Engineered P450 BM3 Variant Panel: Enzyme Family, Name, Sequence, Number of Mutations from Closest Wildtype Parent, and Required Oxidant

Entry	Family	Name ^a	Sequence ^b	Mutations vs. wt	Selection Criteria	Oxidant
1	wt	<u>P450 BM3</u>	Wt	0	fatty acid	O ₂
2	wt	<u>CYP102A2</u>	CYP102A2	0	fatty acid	O ₂
3	wt	<u>CYP102A3</u>	CYP102A3	0	fatty acid	O ₂
4	wt	<u>A1</u>	CYP102A1 Heme Domain Only	0	none	H ₂ O ₂
5	wt	<u>A2</u>	CYP102A2 Heme Domain Only	0	none	H ₂ O ₂
6	wt	<u>wt F87A</u>	wt F87A	1	none	O ₂
7	Propranolol	<u>9C1</u>	21B3 I102T A145V L324I I366V E442K	14	propranolol	H ₂ O ₂
8	Propranolol	<u>D6H10</u>	9C1 L75H V78E A82P	17	propranolol	H ₂ O ₂
9	Propranolol	<u>DE10</u>	9C1 A74V A82L A87G	16	propranolol	H ₂ O ₂
10	Propranolol	<u>2C11</u>	DE10 K24R R47H	18	propranolol	H ₂ O ₂
11	Chimera	11113311	chimera	35	none	H ₂ O ₂
12	Chimera	12112333	chimera	96	none	H ₂ O ₂
13	Chimera	21112233	chimera	98	none	H ₂ O ₂
14	Chimera	21112331	chimera	85	none	H ₂ O ₂
15	Chimera	21112333	chimera	89	none	H ₂ O ₂
16	Chimera	21113312	chimera	97	none	H ₂ O ₂
17	Chimera	21113333	chimera	75	none	H ₂ O ₂
18	Chimera	21212233	chimera	89	none	H ₂ O ₂
19	Chimera	21212333	chimera	87	none	H ₂ O ₂
20	Chimera	21311231	chimera	81	none	H ₂ O ₂
21	Chimera	21311233	chimera	97	none	H ₂ O ₂
22	Chimera	21311311	chimera	63	none	H ₂ O ₂
23	Chimera	21311313	chimera	95	none	H ₂ O ₂
24	Chimera	21311331	chimera	81	none	H ₂ O ₂
25	Chimera	21311333	chimera	81	none	H ₂ O ₂
26	Chimera	21312133	chimera	100	none	H ₂ O ₂
27	Chimera	21312211	chimera	76	none	H ₂ O ₂
28	Chimera	21312213	chimera	99	none	H ₂ O ₂
29	Chimera	21312231	chimera	94	none	H ₂ O ₂
30	Chimera	21312233	chimera	96	none	H ₂ O ₂
31	Chimera	21312311	chimera	76	none	H ₂ O ₂
32	Chimera	21312313	chimera	98	none	H ₂ O ₂
33	Chimera	21312331	chimera	94	none	H ₂ O ₂
34	Chimera	21312332	chimera	83	none	H ₂ O ₂
35	Chimera	21312333	chimera	80	none	H ₂ O ₂
36	Chimera	21313111	chimera	58	none	H ₂ O ₂
37	Chimera	21313231	chimera	96	none	H ₂ O ₂
38	Chimera	21313233	chimera	82	none	H ₂ O ₂
39	Chimera	21313311	chimera	78	none	H ₂ O ₂
40	Chimera	21313313	chimera	84	none	H ₂ O ₂
41	Chimera	21313331	chimera	96	none	H ₂ O ₂
42	Chimera	21313333	chimera	66	none	H ₂ O ₂
43	Chimera	21332233	chimera	61	none	H ₂ O ₂
44	Chimera	22112233	chimera	81	none	H ₂ O ₂
45	Chimera	22112333	chimera	93	none	H ₂ O ₂
46	Chimera	22212333	chimera	88	none	H ₂ O ₂
47	Chimera	22223132	chimera	55	none	H ₂ O ₂

48	Chimera	22311233	chimera	92	none	H ₂ O ₂
49	Chimera	22311331	chimera	98	none	H ₂ O ₂
50	Chimera	22311333	chimera	85	none	H ₂ O ₂
51	Chimera	22312231	chimera	78	none	H ₂ O ₂
52	Chimera	22312233	chimera	79	none	H ₂ O ₂
53	Chimera	22312331	chimera	94	none	H ₂ O ₂
54	Chimera	22312333	chimera	84	none	H ₂ O ₂
55	Chimera	22313231	chimera	92	none	H ₂ O ₂
56	Chimera	22313233	chimera	86	none	H ₂ O ₂
57	Chimera	22313331	chimera	102	none	H ₂ O ₂
58	Chimera	22313333	chimera	70	none	H ₂ O ₂
59	Chimera	23132233	chimera	70	none	H ₂ O ₂
60	Chimera	32312231	chimera	101	none	H ₂ O ₂
61	Chimera	32312333	chimera	53	none	H ₂ O ₂
62	Chimera	32313233	chimera	55	none	H ₂ O ₂
63	Chimera	11113311-R1	chimera	35	none	O ₂
64	Chimera	12112333-R1	chimera	96	none	O ₂
65	Chimera	21113312-R1	chimera	97	none	O ₂
66	Chimera	21113312-R2	chimera	97	none	O ₂
67	Chimera	21311231-R1	chimera	81	none	O ₂
68	Chimera	21311233-R1	chimera	97	none	O ₂
69	Chimera	21313311-R1	chimera	78	none	O ₂
70	Chimera	21333233-R2	chimera	61	none	O ₂
71	Chimera	22132231-R1	chimera	77	none	O ₂
72	Chimera	22223132-R1	chimera	55	none	O ₂
73	Chimera	22312333-R1	chimera	84	none	O ₂
74	Chimera	22313233-R1	chimera	86	none	O ₂
75	Chimera	23132233-R1	chimera	70	none	O ₂
76	Chimera	23132233-R2	chimera	70	none	O ₂
77	Chimera	32312231-R1	chimera	101	none	O ₂
78	Chimera	32312333-R1	chimera	53	none	O ₂
79	Chimera	32313233-R1	chimera	55	none	O ₂
80	Propane	139-3	wt V78A H138Y T175I V178I A184V H236Q E252G R255S A290V A295T L353V	10	octane	O ₂
81	Propane	J	139-3 Y138H I178V F205C S226R A290V	10	propane	O ₂
82	Propane	9-10A	J R47C K94I P142S	13	propane	O ₂
83	Propane	9-10A A328F	9-10A A328F	14	propane	O ₂
84	Propane	9-10A A328M	9-10A A328M	14	propane	O ₂
85	Propane	9-10A A328V	9-10A A328V	14	propane	O ₂
86	Propane	9-10A A78F	9-10A A78F	13	propane	O ₂
87	Propane	9-10A A78S	9-10A A78S	13	propane	O ₂
88	Propane	9-10A A78T	9-10A A78T	13	propane	O ₂
89	Propane	9-10A A82C	9-10A A82C	14	propane	O ₂
90	Propane	9-10A A82F	9-10A A82F	14	propane	O ₂
91	Propane	9-10A A82G	9-10A A82G	14	propane	O ₂
92	Propane	9-10A A82I	9-10A A82I	14	propane	O ₂
93	Propane	9-10A A82L	9-10A A82L	14	propane	O ₂
94	Propane	9-10A A82S	9-10A A82S	14	propane	O ₂
95	Propane	9-10A A82T	9-10A A82T	14	propane	O ₂
96	Propane	9-10A F87A	9-10A F87A	14	propane	O ₂
97	Propane	9-10A F87I	9-10A F87I	14	propane	O ₂
98	Propane	9-10A F87L	9-10A F87L	14	propane	O ₂
99	Propane	9-10A F87V	9-10A F87V	14	propane	O ₂
100	Propane	9-10A L75I	9-10A L75I	14	propane	O ₂

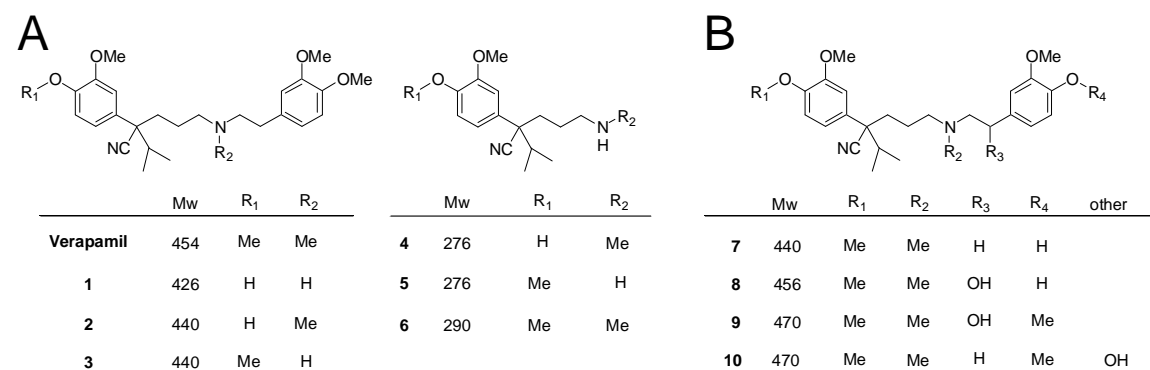
101	Propane	9-10A L75W	9-10A L75W	14	propane	O ₂
102	Propane	9-10A T260L	9-10A T260L	14	propane	O ₂
103	Propane	9-10A T260N	9-10A T260N	14	propane	O ₂
104	Propane	9-10A T260S	9-10A T260S	14	propane	O ₂
105	Propane	9-10A T88L	9-10A T88L	14	propane	O ₂
106	Propane	1-12G	9-10A A82L A328V	15	propane	O ₂
107	Propane	7-11D	9-10A A82F A328V	15	propane	O ₂
108	Propane	11-8E	9-10A F87V A328L	15	propane	O ₂
109	Propane	12-10C	9-10A A82G F87V A328V	16	propane	O ₂
110	Propane	13-7C	9-10A A78T A328V	14	propane	O ₂
111	Propane	23-11B	9-10A A78T A82G F87V A328L	16	propane	O ₂
112	Propane	29-10E	9-10A A82F A328F	15	propane	O ₂
113	Propane	29-3E	9-10A A78F A82G A328F	14	propane	O ₂
114	Propane	35-7F	9-10A A78F A82S A328L	14	propane	O ₂
115	Propane	41-5B	9-10A A78F A82G A328V	14	propane	O ₂
116	Propane	49-1A	9-10A A78T A82G F87V A328L	16	propane	O ₂
117	Propane	49-9B	9-10A A82G F87L A328L	16	propane	O ₂
118	Propane	53-5H	9-10A A78F A82S A328F	15	propane	O ₂
119	Propane	68-8F	9-10A A78F A82G A328L	15	propane	O ₂
120	Propane	77-9H	9-10A A78T A82G A328L	15	propane	O ₂

^a underlined variants contain wildtype sequences, variants in italics were selected for propranolol activity,¹ variants in bold are chimeras,² variants in normal type were selected for activity on alkanes.^{3,4} ^b represented with the parent sequence in bold followed by the mutations in normal type. **21B3** contains the following mutations relative to wildtype: I58V, H100R, F107L, A135S, M145V, N239H, S274T, K434E and V446I. Chimeras are written according to fragment composition: 32313233-R1, for example, represents a protein which inherits the first fragment from parent CYP102A3, the second from CYP102A2, the third from CYP102A3, and so on. The specific amino acid sequence of each block is contained in Supplementary Table 2. R1 connotes the presence of the reductase domain from parent A1, indicating that this chimera is a monooxygenase.

Supplementary Table 2. Amino acid sequence of blocks 1-8 of the cytochrome P450 chimeras

Block	Parent	Sequence
1	A1	-TIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
1	A2	KETSPIPQPKTFGPLGNLPLIDKDKPTLSLIKLAEEQGPIFQIHTPAGTTIVVSGHELVKEVCDE
1	A3	KQASAIQPQPKTYGPLKNLPHLEKEQLSQSLWRIADELGPIFRFDFPGVSSVFVSGHNLVAEVCDE
2	A1	SRFDKNLSQALKFVRDFAGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDI
2	A2	ERFDKSIEGALEKVRAFSGDGLATSWTHEPNWRKAHNILMPTFSQRAMKDYHEKMVDI
2	A3	KRFDKNLGKGLQKVREFGGDGLATSWTHEPNWQKAHRILLPSFSQKAMKGYHSMMLDI
3	A1	AVQLVQKWERLNADEHIEVPEDMTRLTLDITIGLCGFNYRFNSFY
3	A2	AVQLIQKWARLNPNEAVDVPDMDTRLTLDITIGLCGFNYRFNSYY
3	A3	ATQLIQKWSRLNPNEEIDVADDMDTRLTLDITIGLCGFNYRFNSFY
4	A1	RDQPHPFITSMVRALDEAMNKLQRANPDDPAYDENKRQFQEDIKVMNDLV
4	A2	RETPHPFINSMVRALDEAMHQMQRDLVDQDKLMVRTKRQFRYDIQTMFSLV
4	A3	RDSQHPFITSMRLALKEAMNQSKRLGLQDKMMVKTKLQFQKDI EVMNSLV
5	A1	DKIIADRKASGEQ-SDDLTHMLNGKDPETGEPLDDENIRYQIITFLIAGHET
5	A2	DSIIAERRANGDQDEKDLLARMLNVEDPETGEKLDDENIRFQIITFLIAGHET
5	A3	DRMIAERKANPDENIKDLLSLMLYAKDPVTGETLDDENIRYQIITFLIAGHET
6	A1	TSGLLSFALYFLVKNPHVLQKAAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRLWPTAA
6	A2	TSGLLSFATYFLLKHPDKLKKAYEEVDRVLTDAAPTYKQVLELTYIRMILNESLRLWPTA
6	A3	TSGLLSFAIYCLLTHPEKLKKAQEEADRVLTDDTPEYKQIQQLKYIRMVLNETLRLYPTA
7	A1	PAFSLYAKEDTVLGGEYPLEKGDELMVLIPQLHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPF GNGQRACIGQQ
7	A2	PAFSLYPKEDTVIGGKFPITTNDRISVLIPQLHRDRDAWGKDAEEFRPERFEHQDQVPHHAYKPF GNGQRACIGMQ
7	A3	PAFSLYAKEDTVLGGEYPI SKGQPVTVLIPKLHRDQNAWGPDAEDFRPERFEDPSSIPHHAYKPF GNGQRACIGMQ
8	A1	FALHEATLVLGMMMLKHFD FEDHTNYELDIKETLTLKPEGFVVAKSKKIPLGGIPSPST
8	A2	FALHEATLVLG MILKYFTLIDHENYELDIKQTLTLKPGDFHISVQSRHQEAIHADVQAAE
8	A3	FALQEATMVLGLVLKHFELINHTGYELKIKEALT IKPDDFKITVKPRKTAAINVQRKEQA

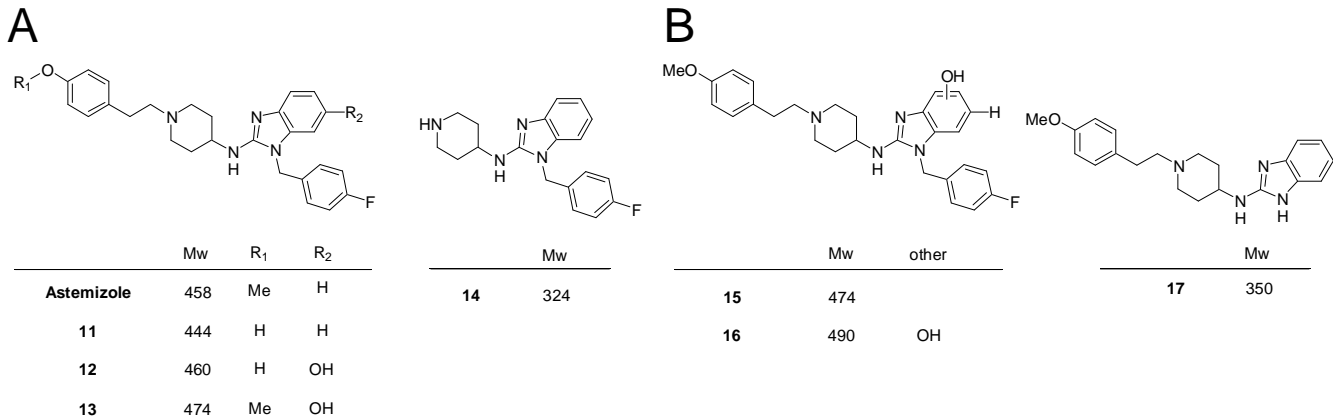
Supplementary Table 3. Complete list of active enzymes and their metabolite distributions with verapamil



Entry	Variant ^a	% Conversion	% 1	% 2	% 3	% 4	% 5	% 6	% 7	% 8	% 9	% 10
1	<i>2C11</i>	25		8	28			20	8		8	28
2	<i>9C1</i>	31	3		29	3		6	13	3	10	13
3	<i>D6H10</i>	78			26	8		31			3	24
4	<i>DE10</i>	20						15	35		10	15
5	11113311	25			48			28	8			16
6	21113312	17		6	12			12	41	6	6	12
7	21113333	5						20	40	20		20
8	21312332	6			17			33	17			33
9	21313111	6						67				33
10	21313311	12			33			33	8			25
11	21313331	5			20			20	20	20		20
12	22313231	43		47				33	5			16
13	22313233	12	8					8	17	8	17	8
14	22313333	34		41			15	21	9			15
15	32313233	24			50			21	13			17
16	23132233-R1	15			27			73				
17	32312231-R1	23			30			43				26
18	32312333-R1	44		7	7				36			
19	32313233-R1	25			40			44				16
20	<i>139-3</i>	14		14	14			14	57			
21	<i>9-10A</i>	27		7	7			7	63	7		
22	<i>L75I</i>	34		6	6			6	74	3		
23	<i>A78F</i>	30		10					83			
24	<i>A78S</i>	20			25			35	35			5
25	<i>A78T</i>	25		8	8			8	64	4		
26	<i>A82C</i>	16			6			6	81			
27	<i>A82F</i>	30			10			3	83			
28	<i>A82G</i>	13			31			46	23			
29	<i>A82I</i>	33		3	6			3	79	3	3	
30	<i>A82L</i>	51						2	94			
31	<i>A82S</i>	32		3	3			6	88			
32	<i>A82T</i>	28		4	7			4	86			
33	<i>F87A</i>	12			8			17	58			
34	<i>F87I</i>	13		8				8	69			
35	<i>F87L</i>	49						6	94			
36	<i>F87V</i>	24	4	4				8	63	4		
37	<i>T88L</i>	14			21			43	21			14
38	<i>T260S</i>	34			12			29	38	9		12
39	<i>A328M</i>	36	3	3	3			6	75		6	
40	<i>1-12G</i>	19			16			21	53			
41	<i>7-11D</i>	21			29			29	14			29
42	<i>12-10C</i>	34			6			6	79	3		
43	<i>41-5B</i>	18			11			6	67			

^a variants in italics were selected for propranolol activity,¹ variants in bold are chimeras,² variants in normal type were selected for activity on alkanes.^{3,4}

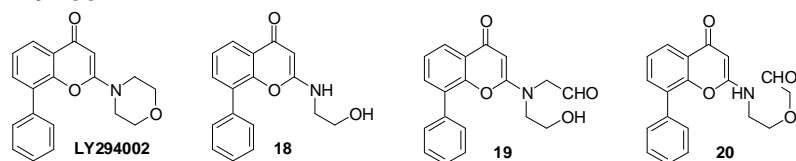
Supplementary Table 4. Complete list of active enzymes and their metabolite distributions with astemizole



Entry	Variant ^a	% Conversion	% 11	% 12	% 13	% 14	% 15	% 16	% 17
1	<i>2C11</i>	4				100			
2	<i>9C1</i>	2				100			
3	<i>DE10</i>	9				56			
4	21113312	6	33			67			
5	21313111	4	25			75			
6	21313311	10	20			40			40
7	22313231	9	22			44			33
8	22313233	7	14			57			29
9	22313333	9	22			56			22
10	32312333	3				100			
11	32313233	11				45	27		27
12	21313111-R1	11	27		27	18	27		
13	22313233-R1	6	33			33			33
14	32312333-R1	78				4	67		9
15	32313233-R1	16				13	69		
16	139-3	6			67		33		
17	J	23	26	4	65			4	
18	9-10A	50	14	10			70	6	
19	L75I	15	33		47				
20	A78F	21	48		38			14	
21	A78S	15	13	7			80		
22	A78T	27	37	7	44			4	
23	A82C	24	33		67				
24	A82F	15	60						
25	A82G	35	29	6	63			3	
26	A82I	32	31	6	63				
27	A82L	49	24				61	4	
28	A82S	30	20	7	70			3	
29	A82T	34	24	12	59			6	
30	F87A	31			23	16	45		
31	F87I	50			48		36	4	
32	F87L	36		3	88			6	
33	F87V	26			38		31		
34	T88L	28			75		25		
35	T260L	2			100				
36	T260N	40		25	75				
37	T260S	25			32	8	44		
38	11-8E	2	100						
39	12-10C	26	38			12	46		
40	23-11B	2	100						
41	41-5B	5	100						
42	49-9B	5	60			40			

^avariants in italics were selected for propranolol activity,¹ variants in bold are chimeras,² variants in normal type were selected for activity on alkanes.^{3,4}

Supplementary Table 5. Complete list of active enzymes and their metabolite distributions with LY294002



Mw:	307	281	323	323		
Entry	Variant ^a	% Conversion	% 18	% 19	% 20	% metabolite A ^b
1	12112333-R1	8				100
2	21113312-R1	8				100
3	21313111-R1	7				100
4	21333233-R1	4				100
5	22132231-R1	7				100
6	32312231-R1	6				100
7	32313233-R1	8				100
8	J	9				100
9	L75I	10		50		
10	A78S	7		71		
11	A82S	2		100		
12	F87V	6			100	
13	T260S	11		9		91
14	A328F	7				100
15	7-11D	7				100
16	29-10E	12				83
17	68-8F	9		11		89
18	77-9H	11				91

^avariants in bold are chimeras,² variants in normal type were selected for activity on alkanes.^{3, 4}

^bMetabolite of unknown structure, Mw=238

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